

10423.204-WO.ST25.txt
SEQUENCE LISTING

<110> Lassen, Soren Flensted
 <120> Improved proteases and methods for producing them
 <130> 10423.204-WO-DK
 <160> 53
 <170> PatentIn version 3.2
 <210> 1
 <211> 1062
 <212> DNA
 <213> Nocardiopsis sp. NRRL 18262

<220>
 <221> misc_feature
 <222> (1)..(495)
 <223> Encodes the pro-region shown in positions -165 to -1 of SEQ ID NO:43.

<220>
 <221> misc_feature
 <222> (496)..(1059)
 <223> Encodes the mature region shown in positions 1-188 of SEQ ID NO:43.

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10423.204-WO.ST25.txt

<210> 2
 <211> 1143
 <212> DNA
 <213> Artificial sequence

<220>
 <223> A synthetic 10R gene (10Rsynt-15) encoding a S2A protease denoted "10R" fused by PCR in frame to the signal peptide encoding sequence of a heterologous protease, Savinase.

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 taa 1143

<210> 3
 <211> 8
 <212> PRT
 <213> Artificial sequence

<220>
 <223> C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 3

Gln Ser His Val Gln Ser Ala Pro
 1 5

<210> 4

10423.204-WO.ST25.txt

<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Polynucleotide encoding a C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 4
caatcgc atg ttcaatccgc tcca

24

<210> 5
<211> 4
<212> PRT
<213> Artificial sequence

<220>
<223> C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 5

Gln Ser Ala Pro
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<210> 6
<211> 12
<212> DNA
<213> Artificial sequence

<220>
<223> Polynucleotide encoding a C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 6
caatcggctc ct

12

<210> 7
<211> 2
<212> PRT
<213> Artificial sequence

<220>
<223> C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 7

Gln Pro
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<210> 8
<211> 6
<212> DNA
<213> Artificial sequence

<220>
<223> Polynucleotide encoding a C-terminal amino acid tail expressed as fusion to protease of the invention.

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6

10423.204-WO.ST25.txt

<210> 9
 <211> 1
 <212> PRT
 <213> Artificial sequence

<220>
 <223> C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 9

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<210> 10
 <211> 3
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Polynucleotide encoding a C-terminal amino acid tail expressed as fusion to protease of the invention.

<400> 10
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3

<210> 11
 <211> 45
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer #252639

<400> 11
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45

<210> 12
 <211> 44
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer #251992

<400> 12
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<210> 13
 <211> 44
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer #179541

<400> 13
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44

<210> 14
 <211> 43
 <212> DNA
 <213> Artificial sequence

10423.204-WO.ST25.txt

<220>
 <223> Primer #179542
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<210> 15
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 <212> DNA
 <213> Artificial sequence

<220>
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 atcc 64

<210> 16
 <211> 60
 <212> DNA
 <213> Artificial sequence

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<210> 17
 <211> 37
 <212> DNA
 <213> Artificial sequence

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 <223> Primer #179154
 <400> 17
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<210> 18
 <211> 37
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer #179153
 <400> 18
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<210> 19
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer #317
 <400> 19
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10423.204-WO.ST25.txt

<210> 20
 <211> 40
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer #139 NotI

<400> 20
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<210> 21
 <211> 7443
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence of plasmid pMB1508

<400> 21
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 ttgcaattga agaattatta atgttaagct taattaaaga taatatcttt gaattgtaac 480
 gccctcaaa agtaagaact acaaaaaaag aatacgttat atagaaatat gtttgaacct 540
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10423.204-WO.ST25.txt

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10423.204-WO.ST25.txt

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10423.204-WO.ST25.txt

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10423.204-WO.ST25.txt

<210> 22
 <211> 5718
 <212> DNA
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<220>
 <223> Sequence of MB1510 genomic integration region

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10423.204-WO.ST25.txt

<210> 23
 <211> 27
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 <213> Artificial sequence

<220>
 <223> Primer 1605

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27

<210> 24
 <211> 42
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer 1606

<220>
 <221> misc_feature
 <222> (13)..(13)
 <223> .n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (16)..(16)
 <223> n is a, c, g, or t

<400> 24
 ccagatctct atnktntgt acggagtcta actccccaag ag

42

<210> 25
 <211> 1112
 <212> DNA
 <213> Nocardiosis dassonvillei DSM 43235

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 gagggccgcgg gcgaggccta cggcggtctca ctgttcgaca ccgagaccct cgaactcacc 240
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10423.204-WO.ST25.txt

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 <211> 48
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer 1423

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<210> 27
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 <213> Artificial sequence

<220>
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<400> 27
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<210> 28
 <211> 354
 <212> PRT
 <213> Nocardiosis dassonvillei DSM 43235

<220>
 <221> PROPEP
 <222> (1)..(166)

<220>
 <221> mat_peptide
 <222> (167)..(354)

<400> 28

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 -150 -145 -140

Ala Glu Ala Glu Glu Leu Leu Ser Ala Gln Glu Ala Ala Ile Glu
 -135 -130 -125

Thr Asp Ala Glu Ala Thr Glu Ala Ala Gly Glu Ala Tyr Gly Gly
 -120 -115 -110

10423.204-WO.ST25.txt

Ser Leu Phe Asp Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp
 -105 -100 -95

Ala Ser Ala Val Glu Ala Val Glu Ala Thr Gly Ala Gln Ala Thr Val
 -90 -85 -80 -75

Val Ser His Gly Thr Glu Gly Leu Thr Glu Val Val Glu Asp Leu Asn
 -70 -65 -60

Gly Ala Glu Val Pro Glu Ser Val Leu Gly Trp Tyr Pro Asp Val Glu
 -55 -50 -45

Ser Asp Thr Val Val Val Glu Val Leu Glu Gly Ser Asp Ala Asp Val
 -40 -35 -30

Ala Ala Leu Leu Ala Asp Ala Gly Val Asp Ser Ser Ser Val Arg Val
 -25 -20 -15

Glu Glu Ala Glu Glu Ala Pro Gln Val Tyr Ala Asp Ile Ile Gly Gly
 -10 -5 -1 1 5

Leu Ala Tyr Tyr Met Gly Gly Arg Cys Ser Val Gly Phe Ala Ala Thr
 10 15 20

Asn Ser Ala Gly Gln Pro Gly Phe Val Thr Ala Gly His Cys Gly Thr
 25 30 35

Val Gly Thr Gly Val Thr Ile Gly Asn Gly Thr Gly Thr Phe Gln Asn
 40 45 50

Ser Val Phe Pro Gly Asn Asp Ala Ala Phe Val Arg Gly Thr Ser Asn
 55 60 65 70

Phe Thr Leu Thr Asn Leu Val Ser Arg Tyr Asn Ser Gly Gly Tyr Gln
 75 80 85

Ser Val Thr Gly Thr Ser Gln Ala Pro Ala Gly Ser Ala Val Cys Arg
 90 95 100

Ser Gly Ser Thr Thr Gly Trp His Cys Gly Thr Ile Gln Ala Arg Asn
 105 110 115

Gln Thr Val Arg Tyr Pro Gln Gly Thr Val Tyr Ser Leu Thr Arg Thr
 120 125 130

Asn Val Cys Ala Glu Pro Gly Asp Ser Gly Gly Ser Phe Ile Ser Gly
 135 140 145 150

Ser Gln Ala Gln Gly Val Thr Ser Gly Gly Ser Gly Asn Cys Ser Val
 155 160 165

10423.204-WO.ST25.txt

Gly Gly Thr Thr Tyr Tyr Gln Glu Val Thr Pro Met Ile Asn Ser Trp
 170 175 180

Gly Val Arg Ile Arg Thr
 185

<210> 29
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 <212> DNA
 <213> Nocardiosis dassonvillei DSM 43235

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<210> 30
 <211> 166
 <212> PRT
 <213> Nocardiosis dassonvillei DSM 43235

<400> 30

Ala Pro Ala Pro Val Pro Gln Thr Pro Val Ala Asp Asp Ser Ala Ala
 1 5 10 15

Ser Met Thr Glu Ala Leu Lys Arg Asp Leu Asp Leu Thr Ser Ala Glu
 20 25 30

Ala Glu Glu Leu Leu Ser Ala Gln Glu Ala Ala Ile Glu Thr Asp Ala
 35 40 45

Glu Ala Thr Glu Ala Ala Gly Glu Ala Tyr Gly Gly Ser Leu Phe Asp
 50 55 60

Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp Ala Ser Ala Val
 65 70 75 80

Glu Ala Val Glu Ala Thr Gly Ala Gln Ala Thr Val Val Ser His Gly
 85 90 95

Thr Glu Gly Leu Thr Glu Val Val Glu Asp Leu Asn Gly Ala Glu Val
 100 105 110

10423.204-WO.ST25.txt

Pro Glu Ser Val Leu Gly Trp Tyr Pro Asp Val Glu Ser Asp Thr Val
 115 120 125

Val Val Glu Val Leu Glu Gly Ser Asp Ala Asp Val Ala Ala Leu Leu
 130 135 140

Ala Asp Ala Gly Val Asp Ser Ser Ser Val Arg Val Glu Glu Ala Glu
 145 150 155 160

Glu Ala Pro Gln Val Tyr
 165

<210> 31
 <211> 1146
 <212> DNA
 <213> Artificial sequence

<220>
 <223> The DNA sequence coding for the pro-region of SEQ ID NO: 29 fused in frame to A1918L2 protease tail-variant encoding gene; whole construct: 10R(proA1918L2).

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agcgccgcca gcatgaccga ggcgctcaag cgcgacctcg acctcacctc ggccgaggcc      180
gaggagcttc tctcggcgca ggaagccgcc atcgagaccg acgccgaggc caccgaggcc      240
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acataa                                           1146

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10423.204-WO.ST25.txt

<210> 32
 <211> 1068
 <212> DNA
 <213> Nocardiosis Alba DSM 15647

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 caggccgagt ccttcgagat cgacgaggcc gccaccgcgg ccgacgccga ctctacggc 180
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<210> 33
 <211> 355
 <212> PRT
 <213> Nocardiosis Alba DSM 15647

<220>
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 <222> (1)..(167)
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 <221> mat_peptide
 <222> (168)..(355)

<400> 33

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 -165 -160 -155

Ala Thr Thr Met Val Glu Ala Leu Gln Arg Asp Leu Gly Leu Ser
 -150 -145 -140

Pro Ser Gln Ala Asp Glu Leu Leu Glu Ala Gln Ala Glu Ser Phe

10423.204-WO.ST25.txt
-130 -125

-135

Glu Ile Asp Glu Ala Ala Thr Ala Ala Ala Ala Asp Ser Tyr Gly
-120 -115 -110Gly Ser Ile Phe Asp Thr Asp Ser Leu Thr Leu Thr Val Leu Val Thr
-105 -100 -95Asp Ala Ser Ala Val Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys
-90 -85 -80Val Val Ser His Gly Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu
-75 -70 -65 -60Asn Ala Ala Asp Ala Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile
-55 -50 -45His Ser Asp Thr Val Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp
-40 -35 -30Val Asp Ser Leu Leu Ala Asp Ala Gly Val Asp Thr Ala Asp Val Lys
-25 -20 -15Val Glu Ser Thr Thr Glu Gln Pro Glu Leu Tyr Ala Asp Ile Ile Gly
-10 -5 -1 1 5Gly Leu Ala Tyr Thr Met Gly Gly Arg Cys Ser Val Gly Phe Ala Ala
10 15 20Thr Asn Ala Ser Gly Gln Pro Gly Phe Val Thr Ala Gly His Cys Gly
25 30 35Thr Val Gly Thr Pro Val Ser Ile Gly Asn Gly Gln Gly Val Phe Glu
40 45 50Arg Ser Val Phe Pro Gly Asn Asp Ser Ala Phe Val Arg Gly Thr Ser
55 60 65Asn Phe Thr Leu Thr Asn Leu Val Ser Arg Tyr Asn Thr Gly Gly Tyr
70 75 80 85Ala Thr Val Ser Gly Ser Ser Gln Ala Ala Ile Gly Ser Gln Ile Cys
90 95 100Arg Ser Gly Ser Thr Thr Gly Trp His Cys Gly Thr Val Gln Ala Arg
105 110 115Gly Gln Thr Val Ser Tyr Pro Gln Gly Thr Val Gln Asn Leu Thr Arg
120 125 130

Thr Asn Val Cys Ala Glu Pro Gly Asp Ser Gly Gly Ser Phe Ile Ser

135

140

10423.204-WO.ST25.txt
145

Gly Ser Gln Ala Gln Gly Val Thr Ser Gly Gly Ser Gly Asn Cys Ser
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<210> 34
<211> 43
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<213> Artificial sequence

<220>
<223> Primer 1421

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43

<210> 35
<211> 31
<212> DNA
<213> Artificial sequence

<220>
<223> Primer 1604

<400> 35
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31

<210> 36
<211> 1062
<212> DNA
<213> Nocardiopsis prasina DSM 15648

<400> 36
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tcggtcggat tcgcggccac caacgccgcc ggtcagcccg gattcgtcac cgccggtcac 600
tgtggccgcg tgggcacca ggtgagcatc ggcaacggcc agggcgtctt cgagcagtcc 660
atcttcccgg gcaacgacgc cgccttcgtc cgcggcacgt ccaacttcac gctgaccaac 720

10423.204-WO.ST25.txt

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ctggtcagcc gctacaacac cggcggttac gccaccgtcg ccggccacaa ccaggcgccc      780
atcggctcct ccgtctgccg ctccggctcc accaccggct ggcactgcgg caccatccag      840
gcccgcggcc agtcggtgag ctaccccgag ggcaccgtca ccaacatgac ccggaccacc      900
gtgtgcgccg agcccggcga ctccggcggc tcctacatct ccggcaacca ggcccagggc      960
gtcacctccg gcggctccgg caactgccgc accggcgggg ccaccttcta ccaggagggtc     1020
accccatgg tgaactcctg gggcgctccgt ctccggacct aa                          1062

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<210> 37
<211> 353
<212> PRT
<213> Nocardiosis prasina DSM 15648

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<220>
<221> PROPEP
<222> (1)..(165)

<220>
<221> mat_peptide
<222> (166)..(353)

<400> 37

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Ala Thr Gly Pro Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala
-165                               -160                               -155

Val Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Gly Leu Thr Pro
-150                               -145                               -140

Leu Glu Ala Asp Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu
-135                               -130                               -125

Val Asp Glu Ala Ala Ala Ala Ala Ala Gly Asp Ala Tyr Gly Gly
-120                               -115                               -110

Ser Val Phe Asp Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp
-105                               -100                               -95                               -90

Ala Ala Ser Val Glu Ala Val Glu Ala Thr Gly Ala Gly Thr Glu Leu
-85                               -80                               -75

Val Ser Tyr Gly Ile Glu Gly Leu Asp Glu Ile Ile Gln Asp Leu Asn
-70                               -65                               -60

Ala Ala Asp Ala Val Pro Gly Val Val Gly Trp Tyr Pro Asp Val Ala
-55                               -50                               -45

Gly Asp Thr Val Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val
-40                               -35                               -30

Ser Gly Leu Leu Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val
-25                               -20                               -15                               -10

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10423.204-WO.ST25.txt

Thr Ser Ser Ala Gln Pro Glu Leu Tyr Ala Asp Ile Ile Gly Gly Leu
 -5 -1 1 5
 Ala Tyr Thr Met Gly Gly Arg Cys Ser Val Gly Phe Ala Ala Thr Asn
 10 15 20
 Ala Ala Gly Gln Pro Gly Phe Val Thr Ala Gly His Cys Gly Arg Val
 25 30 35
 Gly Thr Gln Val Ser Ile Gly Asn Gly Gln Gly Val Phe Glu Gln Ser
 40 45 50 55
 Ile Phe Pro Gly Asn Asp Ala Ala Phe Val Arg Gly Thr Ser Asn Phe
 60 65 70
 Thr Leu Thr Asn Leu Val Ser Arg Tyr Asn Thr Gly Gly Tyr Ala Thr
 75 80 85
 Val Ala Gly His Asn Gln Ala Pro Ile Gly Ser Ser Val Cys Arg Ser
 90 95 100
 Gly Ser Thr Thr Gly Trp His Cys Gly Thr Ile Gln Ala Arg Gly Gln
 105 110 115
 Ser Val Ser Tyr Pro Glu Gly Thr Val Thr Asn Met Thr Arg Thr Thr
 120 125 130 135
 Val Cys Ala Glu Pro Gly Asp Ser Gly Gly Ser Tyr Ile Ser Gly Asn
 140 145 150
 Gln Ala Gln Gly Val Thr Ser Gly Gly Ser Gly Asn Cys Arg Thr Gly
 155 160 165
 Gly Thr Thr Phe Tyr Gln Glu Val Thr Pro Met Val Asn Ser Trp Gly
 170 175 180
 Val Arg Leu Arg Thr
 185

<210> 38
 <211> 43
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer 1346

<400> 38
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43

<210> 39
 <211> 38

10423.204-WO.ST25.txt

<212> DNA

<213> Artificial sequence

<220>

<223> Primer 1602

<400> 39

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38

<210> 40

<211> 1062

<212> DNA

<213> Nocardiosis prasina DSM 15649

<400> 40

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gcgctccagc gcgacctcgg cctgaccccg cttgaggccg atgaactgct ggccgcccag      120
gacaccgcct tcgagggtcga cgaggccgcg gccgaggccg ccggtgacgc ctacggcggc      180
tccgtcttcg acaccgagac cctggaactg accgtcctgg tcaccgactc cgccgcggtc      240
gaggcggtgg aggccaccgg cgccgggacc gaactggtct cctacggcat cacgggcctc      300
gacgagatcg tcgaggagct caacgccgcc gacgccgttc ccggcggtgg cggtggtac      360
ccggacgtcg cgggtgacac cgtcgtgctg gaggtcctgg agggttccgg cgccgacgtg      420
ggcggcctgc tcgccgacgc cggcggtggac gcctcggcgg tcgagggtgac caccaccgag      480
cagcccagac tgtacgccga catcatcggc ggtctggcct acaccatggg cgcccgctgt      540
tcggtcggct tcgcggccac caacgccgcc ggtcagcccg ggttcgtcac cgccggtcac      600
tgtggccgcg tgggcaccca ggtgaccatc ggcaacggcc ggggcgtctt cgagcagtcc      660
atcttcccgg gcaacgacgc cgccttcgtc cgcggaacgt ccaacttcac gctgaccaac      720
ctggtcagcc gctacaacac cggcggttac gccaccgtcg ccggtcacia ccaggcgccc      780
atcggctcct ccgtctgccg ctccggctcc accaccggtt ggcactgcgg caccatccag      840
gcccgcggcc agtcgggtgag ctaccccagag ggcaccgtca ccaacatgac gcggaccacc      900
gtgtgcgccg agcccggcga ctccggcggc tcctacatct ccggcaacca ggcccagggc      960
gtcacctccg gcgggtccgg caactgccgc accggcgggg ccaccttcta ccaggaggtc     1020
accccatggg tgaactcctg gggcggtccgt ctccggacct aa                        1062

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<210> 41

<211> 353

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<213> Nocardiosis prasina DSM 15649

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<222> (1)..(165)

<220>

<221> mat_peptide

<222> (166)..(353)

<400> 41

10423.204-WO.ST25.txt

Ala Thr Gly Pro Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala
 -165 -160 -155
 Val Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Gly Leu Thr Pro
 -150 -145 -140
 Leu Glu Ala Asp Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu
 -135 -130 -125
 Val Asp Glu Ala Ala Ala Glu Ala Ala Gly Asp Ala Tyr Gly Gly
 -120 -115 -110
 Ser Val Phe Asp Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp
 -105 -100 -95 -90
 Ser Ala Ala Val Glu Ala Val Glu Ala Thr Gly Ala Gly Thr Glu Leu
 -85 -80 -75
 Val Ser Tyr Gly Ile Thr Gly Leu Asp Glu Ile Val Glu Glu Leu Asn
 -70 -65 -60
 Ala Ala Asp Ala Val Pro Gly Val Val Gly Trp Tyr Pro Asp Val Ala
 -55 -50 -45
 Gly Asp Thr Val Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val
 -40 -35 -30
 Gly Gly Leu Leu Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val
 -25 -20 -15 -10
 Thr Thr Thr Glu Gln Pro Glu Leu Tyr Ala Asp Ile Ile Gly Gly Leu
 -5 -1 1 5
 Ala Tyr Thr Met Gly Gly Arg Cys Ser Val Gly Phe Ala Ala Thr Asn
 10 15 20
 Ala Ala Gly Gln Pro Gly Phe Val Thr Ala Gly His Cys Gly Arg Val
 25 30 35
 Gly Thr Gln Val Thr Ile Gly Asn Gly Arg Gly Val Phe Glu Gln Ser
 40 45 50 55
 Ile Phe Pro Gly Asn Asp Ala Ala Phe Val Arg Gly Thr Ser Asn Phe
 60 65 70
 Thr Leu Thr Asn Leu Val Ser Arg Tyr Asn Thr Gly Gly Tyr Ala Thr
 75 80 85
 Val Ala Gly His Asn Gln Ala Pro Ile Gly Ser Ser Val Cys Arg Ser
 90 95 100

10423.204-WO.ST25.txt

Gly Ser Thr Thr Gly Trp His Cys Gly Thr Ile Gln Ala Arg Gly Gln
 105 110 115

Ser Val Ser Tyr Pro Glu Gly Thr Val Thr Asn Met Thr Arg Thr Thr
 120 125 130 135

Val Cys Ala Glu Pro Gly Asp Ser Gly Gly Ser Tyr Ile Ser Gly Asn
 140 145 150

Gln Ala Gln Gly Val Thr Ser Gly Gly Ser Gly Asn Cys Arg Thr Gly
 155 160 165

Gly Thr Thr Phe Tyr Gln Glu Val Thr Pro Met Val Asn Ser Trp Gly
 170 175 180

Val Arg Leu Arg Thr
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<210> 42
 <211> 43
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Primer 1603

<400> 42
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43

<210> 43
 <211> 353
 <212> PRT
 <213> Nocardiopsis sp. NRRL 18262

<220>
 <221> PROPEP
 <222> (1)..(165)

<220>
 <221> mat_peptide
 <222> (166)..(1059)

<400> 43

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 -165 -160 -155

Val Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Asp Leu Thr Ser
 -150 -145 -140

Ala Glu Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu
 -135 -130 -125

Val Asp Glu Ala Ala Ala Glu Ala Ala Gly Asp Ala Tyr Gly Gly
 -120 -115 -110

10423.204-WO.ST25.txt

Ser Val Phe Asp Thr Glu Ser Leu Glu Leu Thr Val Leu Val Thr Asp
 -105 -100 -95 -90

Ala Ala Ala Val Glu Ala Val Glu Ala Thr Gly Ala Gly Thr Glu Leu
 -85 -80 -75

Val Ser Tyr Gly Ile Asp Gly Leu Asp Glu Ile Val Gln Glu Leu Asn
 -70 -65 -60

Ala Ala Asp Ala Val Pro Gly Val Val Gly Trp Tyr Pro Asp Val Ala
 -55 -50 -45

Gly Asp Thr Val Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val
 -40 -35 -30

Ser Gly Leu Leu Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val
 -25 -20 -15 -10

Thr Thr Ser Asp Gln Pro Glu Leu Tyr Ala Asp Ile Ile Gly Gly Leu
 -5 -1 1 5

Ala Tyr Thr Met Gly Gly Arg Cys Ser Val Gly Phe Ala Ala Thr Asn
 10 15 20

Ala Ala Gly Gln Pro Gly Phe Val Thr Ala Gly His Cys Gly Arg Val
 25 30 35

Gly Thr Gln Val Thr Ile Gly Asn Gly Arg Gly Val Phe Glu Gln Ser
 40 45 50 55

Val Phe Pro Gly Asn Asp Ala Ala Phe Val Arg Gly Thr Ser Asn Phe
 60 65 70

Thr Leu Thr Asn Leu Val Ser Arg Tyr Asn Thr Gly Gly Tyr Ala Thr
 75 80 85

Val Ala Gly His Asn Gln Ala Pro Ile Gly Ser Ser Val Cys Arg Ser
 90 95 100

Gly Ser Thr Thr Gly Trp His Cys Gly Thr Ile Gln Ala Arg Gly Gln
 105 110 115

Ser Val Ser Tyr Pro Glu Gly Thr Val Thr Asn Met Thr Arg Thr Thr
 120 125 130 135

Val Cys Ala Glu Pro Gly Asp Ser Gly Gly Ser Tyr Ile Ser Gly Thr
 140 145 150

Gln Ala Gln Gly Val Thr Ser Gly Gly Ser Gly Asn Cys Arg Thr Gly
 155 160 165

10423.204-WO.ST25.txt

Gly Thr Thr Phe Tyr Gln Glu Val Thr Pro Met Val Asn Ser Trp Gly
 170 175 180

Val Arg Leu Arg Thr
 185

<210> 44
 <211> 1164
 <212> DNA
 <213> artificial sequence

<220>
 <223> Synthetic protease encoding gene

<220>
 <221> CDS
 <222> (1)..(1164)
 <223> Full length protease

<220>
 <221> sig_peptide
 <222> (1)..(81)

<220>
 <221> misc_feature
 <222> (82)..(1164)
 <223> Propeptide

<220>
 <221> mat_peptide
 <222> (577)..(1164)

<400> 44
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 Met Lys Lys Pro Leu Gly Lys Ile Val Ala Ser Thr Ala Leu Leu
 -190 -185 -180
 att tca gtg gca ttt agc tca tct att gca tca gca gct aca gga 90
 Ile Ser Val Ala Phe Ser Ser Ser Ile Ala Ser Ala Ala Thr Gly
 -175 -170 -165
 gca tta ccg cag tct ccg aca ccg gaa gca gat gca gtc tca atg 135
 Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val Ser Met
 -160 -155 -150
 caa gaa gca ctg caa aga gat ctt gat ctt aca tca gca gaa gca 180
 Gln Glu Ala Leu Gln Arg Asp Leu Asp Leu Thr Ser Ala Glu Ala
 -145 -140 -135
 gaa gaa ctt ctt gct gca caa gat aca gca ttt gaa gtg gat gaa 225
 Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp Glu
 -130 -125 -120
 gca gcg gca gaa gca gca gga gat gca tat ggc ggc tca gtt ttt 270
 Ala Ala Ala Glu Ala Ala Gly Asp Ala Tyr Gly Gly Ser Val Phe
 -115 -110 -105
 gat aca gaa tca ctt gaa ctt aca gtt ctt gtt aca gat gca gca gca 318
 Asp Thr Glu Ser Leu Glu Leu Thr Val Leu Val Thr Asp Ala Ala Ala
 -100 -95 -90
 gtt gaa gca gtt gaa gca aca gga gca gga aca gta ctt gtt tca tat 366
 Val Glu Ala Val Glu Ala Thr Gly Ala Gly Thr Val Leu Val Ser Tyr
 -85 -80 -75

10423.204-WO.ST25.txt

gga att gat ggc ctt gat gaa att gtt caa gaa ctg aat gca gct gat Gly Ile Asp Gly Leu Asp Glu Ile Val Gln Glu Leu Asn Ala Ala Asp -70 -65 -60 -55	414
gct gtt ccg ggc gtt gtt ggc tgg tat ccg gat gtt gct gga gat aca Ala Val Pro Gly Val Val Gly Trp Tyr Pro Asp Val Ala Gly Asp Thr -50 -45 -40	462
gtt gtc ctt gaa gtt ctt gaa gga tca ggc gca gat gtt tca ggc ctg Val Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Ser Gly Leu -35 -30 -25	510
ctg gca gac gca gga gtc gat gca tca gca gtt gaa gtt aca aca tca Leu Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val Thr Thr Ser -20 -15 -10	558
gat caa ccg gaa ctt tat gca gat att att ggc ggc ctg gca tat tat Asp Gln Pro Glu Leu Tyr Ala Asp Ile Ile Gly Gly Leu Ala Tyr Tyr -5 -1 1 5 10	606
atg ggc ggc aga tgc agc gtt ggc ttt gca gca aca aat gca tca ggc Met Gly Gly Arg Cys Ser Val Gly Phe Ala Ala Thr Asn Ala Ser Gly 15 20 25	654
caa ccg ggc ttt gtt aca gca ggc cat tgc ggc aca gtt ggc aca cca Gln Pro Gly Phe Val Thr Ala Gly His Cys Gly Thr Val Gly Thr Pro 30 35 40	702
gtt tca att ggc aat ggc aaa ggc gtt ttt gaa cga agc att ttt ccg Val Ser Ile Gly Asn Gly Lys Gly Val Phe Glu Arg Ser Ile Phe Pro 45 50 55	750
ggc aat gat tca gca ttt gtt aga ggc aca tca aat ttt aca ctt aca Gly Asn Asp Ser Ala Phe Val Arg Gly Thr Ser Asn Phe Thr Leu Thr 60 65 70	798
aat ctg gtt tca aga tat aat tca ggc ggc tat gca aca gtt gca ggc Asn Leu Val Ser Arg Tyr Asn Ser Gly Gly Tyr 85 Ala Thr Val Ala Gly 75 80 90	846
cat aat caa gca ccg att ggc tca gca gtt tgc aga tca ggc tca aca His Asn Gln Ala Pro Ile Gly Ser Ala Val Cys Arg Ser Gly Ser Thr 95 100 105	894
aca ggc tgg cat tgc ggc aca att caa gca aga aat caa aca gtt agg Thr Gly Trp His Cys Gly Thr Ile Gln Ala Arg Asn Gln Thr Val Arg 110 115 120	942
tat ccg caa ggc aca gtt tat agt ctg aca aga aca aca gtt tgt gca Tyr Pro Gln Gly Thr Val Tyr Ser Leu Thr Arg Thr Thr Val Cys Ala 125 130 135	990
gaa ccg ggc gat tca ggc ggc tca tat att agc ggc act caa gca caa Glu Pro Gly Asp Ser Gly Gly Ser Tyr Ile Ser Gly Thr Gln Ala Gln 140 145 150	1038
ggc gtt aca tca ggc ggc tca ggc aat tgc agt gct ggc ggc aca aca Gly Val Thr Ser Gly Gly Ser Gly Asn Cys Ser Ala Gly Gly Thr Thr 155 160 165 170	1086
tat tac caa gaa gtt aat ccg atg ctt agt tca tgg ggc ctt aca ctt Tyr Tyr Gln Glu Val Asn Pro Met Ser Ser Trp Gly Leu Thr Leu 175 180 185	1134
aga aca caa tcg cat gtt caa tcc gct cca Arg Thr Gln Ser His Val Gln Ser Ala Pro 190 195	1164

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<210> 45
<211> 388
<212> PRT
<213> artificial sequence
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<400> 45

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15 20 25

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<220>
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<222> (1) .. (165)
<400> 46
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Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
1 5 10 15
Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Asp Leu Thr Ser Ala Glu
20 25 30
Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp Glu
Page 30

35

10423.204-WO.ST25.txt
40 45Ala Ala Ala Ala Ala Gly Asp Ala Tyr Gly Gly Ser Val Phe Asp
50 55 60Thr Glu Ser Leu Thr Leu Thr Val Leu Val Thr Asp Ala Ser Ala Val
65 70 75 80Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
85 90 95Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
100 105 110Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
115 120 125Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
130 135 140Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val Thr Thr Ser Asp
145 150 155 160Gln Pro Glu Leu Tyr
165<210> 47
<211> 166
<212> PRT
<213> Artificial sequence<220>
<223> Shuffled propeptide G-2.73<220>
<221> PROPEP
<222> (1)..(166)

<400> 47

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
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20 25 30Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp Glu
35 40 45Ala Ala Ala Gly Ala Ala Gly Asp Ala Tyr Gly Gly Ser Val Phe Asp
50 55 60Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp Ala Ser Ala Val
65 70 75 80

10423.204-WO.ST25.txt

Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
85 90 95

Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
100 105 110

Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
115 120 125

Val Val Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
130 135 140

Ala Asp Ala Gly Val Asp Thr Ala Asp Val Lys Val Glu Ser Thr Thr
145 150 155 160

Glu Gln Pro Glu Leu Tyr
165

<210> 48
<211> 166
<212> PRT
<213> Artificial sequence

<220>
<223> shuffled propeptide G-1.43

<220>
<221> PROPEP
<222> (1)..(166)

<400> 48

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
1 5 10 15

Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Gly Leu Ser Ser Ser Gln
20 25 30

Ala Glu Glu Leu Leu Asp Ala Gln Ala Glu Ser Phe Glu Ile Asp Glu
35 40 45

Ala Ala Ala Ala Ala Gly Asp Ala Tyr Gly Gly Ser Ile Phe Asp
50 55 60

Thr Asp Ser Leu Thr Leu Thr Val Leu Val Thr Asp Ala Ser Ala Val
65 70 75 80

Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
85 90 95

Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
100 105 110

10423.204-WO.ST25.txt

Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
 115 120 125

Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
 130 135 140

Ala Asp Ala Gly Val Asp Thr Ala Asp Val Lys Val Glu Ser Thr Thr
 145 150 155 160

Glu Gln Pro Glu Leu Tyr
 165

<210> 49
 <211> 166
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Shuffled propeptide G-2.6

<220>
 <221> PROPEP
 <222> (1)..(166)

<400> 49

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
 1 5 10 15

Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Asp Leu Thr Ser Ala Glu
 20 25 30

Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp Glu
 35 40 45

Ala Ala Ala Ala Ala Gly Asp Ala Tyr Gly Gly Ser Ile Phe Asp
 50 55 60

Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp Ser Ser Ser Val
 65 70 75 80

Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
 85 90 95

Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
 100 105 110

Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
 115 120 125

Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
 130 135 140

10423.204-WO.ST25.txt
 Ala Gly Ala Gly Val Asp Thr Ala Asp Val Lys Val Glu Ser Thr Thr
 145 150 155 160

Glu Gln Pro Glu Leu Tyr
 165

<210> 50
 <211> 165
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Shuffled propeptide G-2.5

<220>
 <221> PROPEP
 <222> (1)..(165)

<400> 50

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
 1 5 10 15

Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Gly Leu Thr Pro Leu Glu
 20 25 30

Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp Glu
 35 40 45

Ala Ala Ala Glu Ala Ala Gly Asp Ala Tyr Gly Gly Ser Val Phe Asp
 50 55 60

Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp Ala Ser Ala Val
 65 70 75 80

Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
 85 90 95

Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
 100 105 110

Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
 115 120 125

Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
 130 135 140

Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val Thr Pro Ala Ala
 145 150 155 160

Arg Pro Glu Leu Tyr
 165

<210> 51

10423.204-WO.ST25.txt

<211> 166
 <212> PRT
 <213> Artificial sequence

<220>
 <223> shuffled propeptide G-2.3

<220>
 <221> PROPEP
 <222> (1)..(166)

<400> 51

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Asp Gly Ala Glu Ala
 1 5 10 15
 Thr Thr Met Val Glu Ala Leu Gln Arg Asp Leu Gly Leu Thr Pro Ala
 20 25 30
 Glu Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp
 35 40 45
 Glu Ala Ala Ala Ala Ala Ala Gly Asp Ala Tyr Gly Gly Ser Ile Phe
 50 55 60
 Asp Thr Asp Ser Leu Thr Leu Thr Val Leu Val Thr Asp Ala Ala Ala
 65 70 75 80
 Val Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His
 85 90 95
 Gly Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp
 100 105 110
 Ala Val Pro Gly Val Val Gly Trp Tyr Pro Asp Val Ala Gly Asp Thr
 115 120 125
 Val Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Tyr Ser Leu
 130 135 140
 Leu Ala Asp Ala Gly Val Asp Ala Ser Ala Val Glu Val Thr Pro Ala
 145 150 155 160
 Ala Gln Pro Glu Leu Tyr
 165

<210> 52
 <211> 166
 <212> PRT
 <213> Artificial sequence

<220>
 <223> shuffled propeptide G-1.4

<220>

10423.204-WO.ST25.txt

<221> PROPEP
 <222> (1)..(166)

<400> 52

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
 1 5 10 15

Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Gly Leu Ser Ser Ser Gln
 20 25 30

Ala Glu Glu Leu Leu Asp Ala Gln Ala Glu Ser Phe Glu Ile Asp Glu
 35 40 45

Ala Ala Ala Ala Ala Ala Ala Asp Ser Tyr Gly Gly Ser Ile Phe Asp
 50 55 60

Thr Asp Ser Leu Thr Leu Thr Val Leu Val Thr Asp Ala Ser Ala Val
 65 70 75 80

Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
 85 90 95

Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
 100 105 110

Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
 115 120 125

Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
 130 135 140

Ala Asp Ala Gly Val Asp Thr Ala Asp Val Lys Val Glu Ser Thr Thr
 145 150 155 160

Glu Gln Pro Glu Leu Tyr
 165

<210> 53
 <211> 166
 <212> PRT
 <213> Artificial sequence

<220>
 <223> shuffled propeptide G-1.2

<220>
 <221> PROPEP
 <222> (1)..(166)

<400> 53

Ala Thr Gly Ala Leu Pro Gln Ser Pro Thr Pro Glu Ala Asp Ala Val
 1 5 10 15

10423.204-WO.ST25.txt

Ser Met Gln Glu Ala Leu Gln Arg Asp Leu Asp Leu Thr Ser Ala Glu
 20 25 30
 Ala Glu Glu Leu Leu Ala Ala Gln Asp Thr Ala Phe Glu Val Asp Glu
 35 40 45
 Ala Ala Ala Ala Ala Ala Gly Asp Ala Tyr Gly Gly Ser Ile Phe Asp
 50 55 60
 Thr Glu Thr Leu Glu Leu Thr Val Leu Val Thr Asp Ser Ser Ser Val
 65 70 75 80
 Glu Ala Val Glu Ala Ala Gly Ala Glu Ala Lys Val Val Ser His Gly
 85 90 95
 Met Glu Gly Leu Glu Glu Ile Val Ala Asp Leu Asn Ala Ala Asp Ala
 100 105 110
 Gln Pro Gly Val Val Gly Trp Tyr Pro Asp Ile His Ser Asp Thr Val
 115 120 125
 Val Leu Glu Val Leu Glu Gly Ser Gly Ala Asp Val Asp Ser Leu Leu
 130 135 140
 Ala Gly Ala Gly Val Asp Thr Ala Asp Val Lys Val Glu Ser Thr Thr
 145 150 155 160
 Glu Gln Pro Glu Leu Tyr
 165